REMARKS

Claims 1, 4, 12, 13, and 16-20 are pending. Claims 1, 4, 12, 13, and 16-20 stand rejected under 35 U.S.C. § 112, first paragraph, and claim 17 is objected to. Applicants address each of these rejections as follows.

Claim Amendments

Claim 18 has been canceled and new claims 21-24 have been added. New claims 21 and 22 find support, for example, at page 85, lines 11-18, and page 89, lines 4-18, of the specification, and new claims 23 and 24 find support, for example, at page 25, lines 2-6, of the specification and in original claims 1 and 17. No new matter has been added by these amendments.

Claim Objection

The Office objects to claim 17 for reciting the phrase "a gene that hybridizes under stringent conditions to SEQ ID NO:54" as SEQ ID NO:54 is an amino acid sequence. Claim 17, as amended, recites "a gene that hybridizes under highly stringent conditions to the complement of a *nucleic acid sequence encoding* the sequence of SEQ ID NO:54." As the claim now requires hybridization between two nucleic acid sequences, Applicants submit that this objection should be withdrawn.

Rejection under 35 U.S.C. § 112, first paragraph

Claims 1, 4, 12-13, and 16-20 stand rejected under 35 U.S.C. § 112, first paragraph, for an asserted lack of written description in the specification and for an asserted lack of enablement. These rejections are respectfully traversed.

Written Description

With respect to the written description rejection, the Office states (page 3, page 5):

> Besides the amino acid sequences of SEQ ID NO:54 the specification as filed fails to disclose any variant of SEQ ID NO:54 that functions in insulin signaling.

Applicants disagree.

Applicants submit that their specification provides a written description of the presently claimed invention in sufficient detail to satisfy the standard set forth by the Patent Office is its Written Description Guidelines and by the Federal Circuit in *Lilly*. In particular, *Lilly* specifically states that the written description of a genus of DNA may be achieved by a "recitation of structural features common to members of the genus." *Regents of University of California v. Eli Lilly & Co.*, 119 F.3d 1159, 43 U.S.P.Q.2d 1398 (Fed. Cir. 1997). Moreover, the Guidelines for Examination of Patent Applications Under 35 U.S.C. 112 ¶1, "Written Description" Requirement, 66 Fed. Reg. 1099 (Jan. 5, 2001) similarly state:

> The written description requirement for a claimed genus may be satisfied ... by disclosure of relevant, identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by

> a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus.

Applicants' specification meets this standard for the presently claimed invention.

First, the specification *does* set forth variants of SEQ ID NO:54 that function in insulin signaling. The specification, for example, in Figure 21A discloses the human FKHR and AFX sequences and shows an alignment between DAF-16 (including SEQ ID NO:54) and these human sequences. In view of this Figure, one skilled in the art would recognize that the human FKHR and AFX sequences are highly similar to the sequence of SEQ ID NO:54. The specification goes on to teach (page 55, lines 22-27):

> As shown in Figs. 21A-21B, the human FKHR and AFX genes, identified as oncogene breakpoints but not as insulin signaling genes, are much more closely related to DAF-16 than the next closest relative in either Genbank or in the 94% complete *C. elegans* genome sequence. These data indicate that FKHR and AFX are excellent candidates for subserving the same function as *C. elegans* DAF-16: transduction of insulin signals and convergence with DAF-7-like Smad signals. (emphasis added)

Thus, Applicants' specification discloses variants of SEQ ID NO:54 that one skilled in the art would expect to function in insulin signaling.

Moreover, inventor Dr. Gary Ruvkun has demonstrated that at least one of these variants can functionally substitute for *daf-16 in vivo*. In the Ruvkun Declaration submitted with Applicants' July 24, 2003 reply, it is stated (paragraph 4):

> We have shown that when a *daf-16* human homolog, FKHRL1, was expressed under the control of the *daf16β* promoter in worms having mutations in *daf-16* and *daf-2*, the human protein was able to replace the worm DAF-16 protein, although the human protein's ability to rescue the *daf-16* phenotype (70%) was somewhat weaker than that of a *C. elegans* DAF-16 protein (100%). These results prove that the human and *C. elegans* proteins are orthologs. Other highly similar DAF-16 family members

would also be expected to substitute for *C. elegans* DAF-16. (emphasis added; citation omitted)

Thus, as is taught in Applicants' specification, FKHR can serve the same function as DAF-16.

In view of these experiments, there can be no question that Applicants' specification satisfies the written description requirement. Applicants describe polypeptide sequences homologous to *daf-16*. They identify an important structural domain – SEQ ID NO:54, as required by Applicants' claims. And they state and later demonstrate the functional relatedness of these sequences by showing that one can substitute for the other functionally *in vivo*.

Consistent with this, Applicants' present claims require the use of a gene that encodes a polypeptide having at least 85% homology to SEQ ID NO:54 and that functions in insulin signaling. This degree of sequence similarity is more than adequate for distinguishing a genus of functional polypeptides as demonstrated by Applicants' experimental results. As noted above, FKHR can functionally substitute for DAF-16, and FKHR is only approximately 71% identical to the sequence of SEQ ID NO:54, as shown by the alignment in Figure 21A. Thus, Applicants have demonstrated that a sequence that is *less* homologous than required by the present claims can functionally substitute for DAF-16 in insulin signaling. Applicants submit that the degree of sequence identity required by claim 1 is therefore a recitation of a structural feature common to members of the genus. Moreover, the claim requires that the gene encodes a polypeptide that functions in insulin signaling. Claim 1 therefore satisfies the written description

requirement by not only defining the claimed gene by an identifying structural feature, but also by functional characteristics that are shared by genus members. The written description rejection of claim 1 and its dependent claims should be withdrawn.

Hybridization

With regard to claim 17, and its dependent claims, Applicants submit that these claims also clearly satisfy the written description requirement. On this issue, Applicants direct the Office's attention to Example 9:Hybridization of the U.S. Patent & Trademark Office's Written Description Guidelines (http://www.uspto.gov/web/menu/written.pdf; "the Guidelines"). In this Example, the Guidelines provide a fact pattern where a single cDNA species that encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase is disclosed in the specification. The claim, in Example 9 of the Guidelines, is directed to a genus of nucleic acids all of which must hybridize under highly stringent conditions with the disclosed cDNA and must encode a protein with a particular activity. In concluding that the written description requirement was satisfied in this Example, the Guidelines state:

> [A] person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because the highly stringent hybridization conditions set forth in the claim yield structurally similar DNAs. Thus, a representative number of species is disclosed, since highly stringent hybridization conditions in combination with the coding function of DNA and the level of skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention. (emphasis added)

The facts of the present case are squarely within these Guidelines and, in particular, parallel Example 9 quoted above. Applicants' current claim 17 encompasses a nucleic acid molecule that specifically hybridizes to the complement of the sequence set forth in SEQ ID NO:54 under highly stringent conditions. (These conditions are described, for example, at page 78, line 21, to page 79, line 2, of the specification.) Claim 17 also requires the nucleic acid molecule to encode a polypeptide that functions in insulin signaling. As in Example 9 of the Guidelines, Applicants' specification, therefore, describes (i) at least a single species of a nucleic acid molecule falling within the scope of the claimed genus (and in fact describes more than a single species) and (ii) an activity of the protein encoded by the nucleic acid molecule (i.e., insulin signaling). A person of ordinary skill in the art would not expect substantial variation among species encompassed within the scope of the present claims. The highly stringent hybridization requirement in claim 17 limits the claim to structurally similar nucleic acids which, when combined with the functionality requirement, describes a genus of nucleic acid molecules that are well within the written description requirement. The written description rejection of claim 17 and its dependent claims should also be withdrawn.

Description of FKHR and AFX

As a further basis for the written description rejection, the Office also asserts (page 3):

> [T]he specification as filed fails to identify the relevant characteristics such that a person skilled in the art would recognize the human FKHR and

human AFX genes. For example the specification fails to disclose an amino acid sequence identified by an SEQ ID NO for human FKHR and AFX.

This basis for the rejection is respectfully traversed. Applicants' specification provides a more than adequate written description of these genes and their use in Applicants' screening method.

First, the specification, for example, at page 85, lines 11-18, and page 89, lines 4-18, describes use of human DAF-16 homologs in screening methods for evaluating whether a test compound is effective in inhibiting DAF-16 gene activity, and, for example, at page 55, lines 25-27, teaches that FKHR and AFX are excellent candidates for serving the same function as *C. elegans* DAF-16. In Figure 21A, the specification sets forth an alignment between *C. elegans* DAF-16 and FKHR and AFX.

Moreover, contrary to the Office's assertion, Applicants need not disclose the amino acid sequences of these polypeptides in their specification, as the sequences of these human genes were known in the art at the time of filing. As evidence of this assertion, the Office is directed to GenBank Accession Number U02310 (creation date 28 December 1993; copy enclosed as Exhibit 1); and UniProt Entry P98177 and the abstract of Borkhardt et al. ("Cloning and Characterization of AFX, the Gene that Fuses to ML in Acute Leukemias with a t(x;11)(q13;q23)," Oncogene 14(2):195-202, January 16, 1997; copies enclosed as Exhibits 2A and 2B)). These references demonstrate the public availability of the human FKHR and human AFX sequences prior to Applicants' filing date. Applicants need not provide in their specification what is publicly available to satisfy the written description requirement.

This final basis for the rejection should also be withdrawn.

Enablement

Claims 1, 4, 12-13, and 16-20 stand further rejected as lacking enablement. This rejection is respectfully traversed.

Applicants' present claims are directed to methods for identifying a candidate modulatory compound for ameliorating or delaying an impaired glucose tolerance condition. These methods involve contacting a *C. elegans* or an isolated *C. elegans* cell expressing either (i) a gene encoding a polypeptide having at least 85% homology to SEQ ID NO:54 (claim 1), (ii) a gene that hybridizes under highly stringent conditions to the complement of a nucleic acid sequence encoding SEQ ID NO:54 (claim 17), (iii) the human FKHR gene (claim 21), or (iv) the human AFX gene (claim 22) with a candidate compound, and monitoring expression or activity of these genes. In the claimed methods, a decrease in expression or activity following contact of the *C. elegans* or the isolated *C. elegans* cell with the candidate compound identifies the compound as a candidate for ameliorating or delaying an impaired glucose tolerance condition.

These screening methods are enabled by the teachings of the present specification.

DAF-16 isoforms

As the first basis for the enablement rejection, the Office states (page 7):

> [W]ithin the Fork head DNA-binding domain, DAF-16a is 65% and 62% identical to FKHR and AFX, whereas DAF-16b is 50% and 47% identical

> to FKHR and AFX genes. In addition the molecular analysis of other daf-16 mutant alleles revealed that the two major DAF-16 isoforms are not redundant ...Thus considering the scope of the instant invention as claimed it is considered highly unpredictable that a 5% variation (85% identical) [*sic*] or any hybridization product obtained form [*sic*] any organism would encode insulin signaling like activity, which is similar to *C. elegans* daf-16 gene.

Applicants respectfully disagree. Applicants have identified differentially spliced isoforms for *daf-16* but, as is taught, for example, at page 53, lines 23-24, of the specification, the data suggest that "both DAF-16 isoforms are necessary for metabolic control." Whether or not the DAF-16 isoforms are redundant is irrelevant. The DAF-16 sequence recited in the present claims, SEQ ID NO:54, is part of a protein that Applicants have shown to be involved in a signaling pathway that parallels the human insulin-signaling pathway. Whether one DAF-16 or two DAF-16 polypeptides are involved in *C. elegans* does not call into question the validity of the screening assay.

Moreover, with respect to the level of sequence identity required by the present claims, as noted above, the Ruvkun Declaration establishes that human FKHR can functionally substitute for DAF-16 in *C. elegans*. Thus, Applicants have clearly established that a highly related human gene (which, in fact, is less than 85% identical) can function to provide insulin-signaling activity that is similar to *C. elegans* DAF-16. This basis for the rejection should be withdrawn.

Impaired Glucose Tolerance Conditions

In asserting a lack of enablement, the Office also seems to question whether DAF-16 is an appropriate target for identifying candidate modulatory compounds for impaired glucose tolerance conditions. On this point there can be no dispute. As indicated in the present specification, Applicants have identified DAF-16 as a gene involved in an insulin signaling-like pathway in *C. elegans*. This discovery by Applicants has been accepted as a sound scientific result, and the connection between DAF-16 and an insulin signaling-like pathway is now well established in the art. In addition, a role in insulin sensitivity for both FKHR and AFX has also been recognized in the art. On these points, Applicants direct the Office's attention, for example, to the attached publications from third parties acknowledging Applicants' discovery. Exhibit 3, for example, a reference by Tsai et al. ("Insulin Inhibition of Transcription Stimulated by the Forkhead Protein Foxo1 Is not Solely Due to Nuclear Exclusion," Endocrinology 144(12):5615-5622, 2003) states (page 5615):

> Insulin inhibition of IGFBP-1 gene expression is mediated by phosphatidylinositol 3-kinase (PI 3-kinase) and its downstream effector, serine/threonine-specific protein kinase B (PCB)/Akt. Recognition that a similar insulin signaling pathway in *Caenorhabditis elegans* inhibited the transcription factor Daf-16, an ortholog of the FOXO subfamily of forkhead transcription factors, suggested that FOXO proteins might mediate insulin inhibition of transcription in mammalian cells. Three human FOXO proteins (FOXO1 [FKHR], FOXO3a [FKHRL1], and FOXO4 [AFX]) and their mouse counterparts (Foxo1, Foxo3, and Foxo4) have been identified and extensively characterized. They share a conserved central DNA-binding domain ... The FOXO proteins bind to an IRE [Insulin Response Element] in the proximal promoter of target genes involved in insulin sensitivity. (citations omitted)

Similarly, Exhibit 4, a reference by Cahill et al. (Phosphatidylinositol 3-Kinase Signaling Inhibits DAF-16 DNA Binding and Function via 14-3-3-Dependent and 14-3-3-Independent Pathways," J. Biol. Chem. 276:13402-13410, 2001) states (page 13402, right column):

> [I]dentifying the downstream targets of insulin signaling to the nucleus has focused on the role mammalian homologues of DAF-16, FKHR, FKHRL1, and AFX in mediating the negative effect of insulin/IGF-1 signaling on gene transcription.

In yet another example of the acceptance of Applicants' discovery by those of skill in the art, Exhibit 5, a reference by Kamei et al. ("Skeletal Muscle FOXO1 (FKHR) Transgenic Mice Have Less Skeletal Muscle Mass, Down-Regulated Type I (Slow Twitch/Red Muscle) Fiber Genes, and Impaired Glycemic Control," J. Biol. Chem. 279:41114-41123, 2004) states (page 41115, left column):

> [A] genetic study of *Caenorhabditis elegans* showed that DAF16, the worm counterpart of FOXO, functions as a suppressor of insulin receptor-like signaling. Thus, the FOXO family [FKHR, FKHRL1, AFX] may act negatively in mammals as a downstream player in insulin or IGF signaling.

Thus, the art published after Applicants' filing date supports what is taught in the specification: namely, that DAF-16 is involved in metabolic control, including impaired glucose tolerance conditions. This fact is clearly recognized and accepted by those of skill in the art. This basis for the enablement rejection should be withdrawn.

Atherosclerosis and Obesity

Finally, new claims 23 and 24 have been added to highlight the fact that atherosclerosis and obesity are well established in the art as being impaired glucose tolerance conditions. As noted in the specification at page 25, lines 2-6, impaired glucose tolerance conditions include Type I diabetes, Type II diabetes, and gestational diabetes, and may be associated with obesity and atherosclerosis.

The Office appears to question the connection between impaired glucose tolerance and both obesity and atherosclerosis, stating (page 8):

> The state of the art regarding arteriosclerosis teaches that development of the arteriosclerosis is complex and several factors like smoking, diet and exercise, hypercholesterolemia, hypertension, diabetes, and some genetic factors account for much less than 100% of disease.
>
> * * *
>
> Similarly obesity is a complex phenotype which is not only the result of genetic variations by is also the out come of personal behavioral and life style.

The Office also cites O'Connor et al. (Potential Infectious Etiologies of Atherosclerosis: A Multifactorial Perspective," Emerging Infectious Diseases 7:780-788, 2001), Lönnqvist et al. ("Overexpression of the Obese (*ob*) gene in Adipose Tissue of Human Obese Subjects," Nat. Med. 1:950-953, 1995), and Kahn et al. ("Obesity, Body Fat Distribution, Insulin Sensitivity and Islet β-Cell Function as Explanations for Metabolic Diversity," J. Nutr. 131:354S-360S, 2001; hereafter "Kahn") in support of the above statements.

The references cited by the Office, while indicating that the development of atherosclerosis and obesity is complex, do not indicate that the presently claimed methods

for identifying candidate compounds for ameliorating or delaying an impaired glucose tolerance condition would be ineffective. In fact, the link between impaired glucose tolerance and atherosclerosis and obesity is well established. For example, Kahn (the reference cited by the Office) teaches (page 354S, left column):

> The prevalence of obesity in the United States is increasing dramatically. This increase in the prevalence of obesity appears to be associated with an increased prevalence of risk factors for cardiovascular disease and type 2 *diabetes*, including hypertension and *reduced glucose tolerance* (citations omitted; emphasis added).

Similarly, Dominiczak ("Obesity, Glucose Intolerance and Diabetes and Their Links to Cardiovascular Disease. Implications for Laboratory Medicine," Clin. Chem. Lab. Med. 41(9):1266-1278, 2003; copy enclosed as Exhibit 6) states (abstract):

> Glucose intolerance and diabetes increase the risk of atherosclerotic events. Moreover, obesity, and glucose intolerance or diabetes, are components of the metabolic syndrome, which also imparts an increased cardiovascular risk.

Further Sinha et al. ("Prevalence of Impaired Glucose Tolerance Among Children and Adolescents with Marked Obesity," N. Engl. J. Med. 346:802-810, 2002; copy enclosed as Exhibit 7) states (page 802, left column):

> In adults, type 2 diabetes develops over a long period, and most if not all, patients have impaired glucose tolerance, which is an intermediate stage in the natural history of type 2 diabetes and predicts the risk of the development of diabetes and cardiovascular disease.

The above references support the well-established connection between impaired glucose tolerance and atherosclerosis and obesity. Further, as indicated above, the role of *daf-16* and *daf-16*-like genes in insulin signaling and glucose intolerance is well established in

the art. Accordingly, Applicants submit that compounds identified in the present screening methods are candidate compounds for ameliorating or delaying an impaired glucose tolerance condition such as atherosclerosis or obesity, as required by the present claims. The enablement rejection of the present claims should be withdrawn.

CONCLUSION

Applicants submit that the application is in condition for allowance, and this action is hereby respectfully requested. Enclosed are a Petition to extend the period for replying to the Office Action for three months, to and including February 25, 2005, and a check in payment of the required extension fee.

The undersigned hereby respectfully requests a telephonic interview with the Examiner to discuss the arguments set forth in the present reply once the Examiner has reviewed these arguments.

If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 25 February 2005

Karen L. Elbing, Ph.D.
Reg. No. 35,238

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045